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DEPARTMENT OF HEALTH AND HUMAN SERVICES

NOTE TO THE FILE

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Subject: Glufosinate Tolerant Corn

Keywords:
Corn, *Streptomyces viridochromogens*, Glufosinate ammonium tolerant, Herbicide tolerant, *pat*, Phosphinothricin Acetyltransferase (PAT), Liberty Link™.

Background

In a submission dated 29 August 1995, as amended on 28 October 1995, AgrEvo USA Company provided summary information to support their safety and nutritional assessment of their new corn lines containing transformation events T14 and T25.

Intended Effect and Food/Feed Use

The intended technical effect of this genetic modification of corn plants is to confer tolerance to the phosphinothricin herbicide glufosinate ammonium. Corn grain (kernels) are primarily used for animal feed and human food. The foliar parts of corn plants are primarily used for the production of silage which is used in animal feed. Corn oil, corn syrup, and cornmeal are the primary by-products of corn grain that are used in human foodstuffs. Corn oil is commonly used as a vegetable oil in human food. Corn syrup is used primarily as a sweetener in human food. Corn by-products used in animal feed include: corn gluten feed and meal; corn germ meal; and hominy feed.

According to AgrEvo, their T14 and T25 containing corn lines have been modified to express a synthetic version of the *pat* gene, similar to the *pat* gene isolated from *Streptomyces viridochromogenes*. The *pat* gene encodes the phosphinothricin acetyltransferase (PAT) protein, which reportedly confers tolerance to the herbicide glufosinate ammonium.

Molecular Alterations and Characterization

A polyethylene glycol-mediated protoplast transformation method was used by AgrEvo to generate the transformation events T14 and T25. The intact circular transformation vector, pUC/Ac, was constructed by inserting a synthetic *pat* gene into the *Sal*1 site, between the CaMV 35S promoter and terminator sequences, of plasmid pDH51. According to AgrEvo, PUC/Ac contains two open

reading frames, *amp*^{r} (under the control of bacterial expression signals) and *pat*.

Based on Southern and Polymerase Chain Reaction (PCR) analyses of total DNA isolated from transgenic corn lines, containing transformation events T14 and T25, and the parental line, AgrEvo has concluded that their corn lines with transformation events T14 and T25 contain 3 and 1 copies of the *pat* gene, and 4 and 1 copies of the *amp*^{r} gene, respectively. AgrEvo states that "[d]espite the multiple integration [in T14], the tolerance trait segregates as a single locus, suggesting that only one copy of the *pat* gene is functional." Moreover, AgrEvo states that gene expression assays verify that the PAT protein is present in leaf, root, and seed tissues, but not present in pollen derived from T14 and T25.

Additionally, AgrEvo reported that PCR analysis with primers specific to portions of the *amp*^{r} gene demonstrates that T25 contains only the 5′ end of the *amp*^{r} gene; and that T14 does not contain any intact *amp*^{r} gene. The firm also conducted enzyme activity assays and Northern analysis of RNA transcripts from T14 and T25 and concluded that the *amp*^{r} gene is not expressed.

AgrEvo reports that they engineered the *pat* gene to optimize its expression in plants. Despite differences in the DNA sequences, the synthetic *pat* gene, introduced by transformation events T14 and T25, codes for an enzyme identical to the PAT protein expressed in *S. viridochromogenes*.

Based on Southern analysis, AgrEvo concluded that the restriction pattern of the integrated DNA is stably inherited in all generations they have examined. Moreover, based on segregation analysis of the glufosinate tolerant phenotype, AgrEvo concluded that the glufosinate tolerant trait is stably inserted and transmitted to progeny as a single dominant gene.

Expressed Protein

Based on DNA analysis of T14 and T25, the only new protein expected to be expressed in corn lines T14 and T25 is PAT. PAT was not expected to be a component of corn oil, and the firm reports that no PAT activity was detected. However, PAT may be present in other products derived from the corn kernel and vegetative tissues.

AgrEvo examined the *in vitro* heat stability and pH optima of PAT, reporting that the temperature for maximum PAT activity is 60°C, and the enzyme loses 100% of its activity upon incubation at 75°C or greater for 30 minutes. According to AgrEvo, the pH optimum for PAT activity is between Ph 7.5 and 8.0. No PAT activity was

reported after incubation for 30 minutes or more at pH values less than or equal to pH 4.

AgrEvo determined the amount of PAT protein by ELISA in corn plants harvested at the forage, silage, and fodder stages, and in grain derived from T14 and T25. AgrEvo found that the PAT protein represents up to 0.00060%, 0.000039%, and 0.00001% of the crude protein in silage, fodder, and grain, respectively. AgrEvo concluded that both the ensiling process and the heat treatments used for processing grain should eliminate most PAT activity.

AgrEvo reported confirming experimentally that PAT protein and *pat* DNA in glufosinate tolerant canola are degraded *in vitro* by the digestive fluids of swine, chicken, and cattle. AgrEvo contends that these results can be extended to the transgenic corn lines, as comparable molecular size PAT proteins are expressed in canola, corn, and bacteria. AgrEvo also reported that the PAT protein is degraded and inactivated in simulated human gastric fluids within minutes.

AgrEvo also characterized the substrate specificity of PAT for L-phosphinothricin, by examining the competitive influence of L-glutamate, other amino acids, and related compounds.

In summary, Agrevo concluded that:

> the PAT protein is inactivated by high temperatures or extremes in Ph. The enzyme displays kinetics typical of those found in the plant kingdom. This highly specific enzyme catalyzes the acetylation of [phosphinothricin], while not affecting L-glutamate or other amino acids. In addition, the enzyme and DNA are rapidly inactivated by gastric juices. Collectively these results indicate that the enzymatic properties of PAT protein and *pat* gene should not raise any safety concerns.

Allergenic and Toxic Potential

AgrEvo argues that while PAT has a molecular mass in the range of known food allergens, PAT does not share any other physical characteristics common to protein allergens. According to AgrEvo, PAT is not heat or acid stable, is not glycosylated, and loses enzyme activity or ELISA reactivity during ensiling and grain processing. In addition, AgrEvo notes that PAT enzyme is extremely labile to digestion in simulated human and animal digestive fluids. They further noted that because PAT is not expressed in the pollen from T14 and T25, inhalation of corn pollen would not serve as an avenue of exposure to the PAT protein. AgrEvo also compared the synthetic gene sequence and the amino acid sequence of PAT, as expressed in T14 and T25, with the sequences reported in the EMBL and SWISSPROT databases, respectively. They reported that none of the sequences with

which PAT shares homology are known allergens or toxins.

AgrEvo concludes that regardless of the level of PAT protein, there is no evidence to support that the PAT protein should pose any significant toxic or allergenic risk to consumers of food derived from T14 and T25 corn.

Nutritional Assessment

Grain

Based on the nature of the genetic modification, it is expected that T14 and T25 corn would not materially differ in composition from other corn varieties. To confirm this expectation, AgrEvo analyzed the nutrient composition of grain obtained from T14 and T25 corn and comparable control lines by standard methods for moisture, crude fat, crude protein, crude fiber, ash, carbohydrate, amino acids, and fatty acids.

No statistically significant differences were reported by AgrEvo in the levels of protein, fiber, and ash between grain derived from T14 and T25 and non-transformed control plants. AgrEvo reported that they did observe statistically significant differences in fat and carbohydrate contents between T14 and T25 corn genotypes and their controls, but T14 and T25 values fell within the ranges reported in the literature for corn.

AgrEvo also reported measuring no differences in the levels of 15 of 18 amino acids, but found statistically significant increases in the amounts of three amino acids (arginine, histidine, and lysine), in the kernels of T14 and T25 genotypes when compared to their control plants. AgrEvo concluded that these differences were small and do not reflect a material alteration in the overall amino acid profile of grain derived from T14 and T25.

Fatty Acids

AgrEvo reported that, of the most abundant fatty acids present in the corn test plants, three (stearic, linolenic, and arachidic) were statistically different in T14 and T25 when compared to the controls. However, the reported values do not appear meaningfully different and the firm reports that the qualitative and quantitative profiles of total lipid and the fatty acids were similar to values reported for grain.

Vegetative Tissues

The likelihood of the PAT protein becoming a macro-constituent in animal diets was examined by AgrEvo. Specifically, PAT concentration was assessed in field grown corn plants at the forage, silage, and fodder stages and on mature grain. AgrEvo reported that a small amount of PAT protein (< 0.0007% of crude

protein) is present in the silage, fodder, and grain derived from transformation events T14 and T25. AgrEvo states that regardless of the level present, PAT is not likely to be toxic or allergenic, and is readily digested and/or degraded in the consumer's gut.

Compositional analyses of green corn forage with ears, described as silage in the submission, included crude protein, crude fat, moisture, acid detergent fiber (ADF), neutral detergent fiber (NDF), ash, and carbohydrate by calculation. AgrEvo reports that transgenic lines differed significantly from controls in concentrations of crude fat, crude protein, ADF, and NDF. However, the firm reports that despite these differences, values fell within literature ranges reported for ensiled material. AgrEvo concluded that the nutrient composition of "silage" derived from T14 and T25 did fall within ranges determined from commercial varieties of corn.

AgrEvo also examined the level of the antinutrient phytic acid in "silage" derived from T14 and T25. They concluded that there is no statistically significant difference in phytic acid content between T14 and T25 and non-transformed control plants.

AgrEvo indicated that inclusion of the novel genetic material in corn did not affect hybrid susceptibility to attack by fungal organisms.

Conclusions

AgrEvo has concluded that corn lines containing transformation events T14 and T25 are not materially different in composition, nutrition, and safety from corn currently grown, marketed, and consumed for animal feed or human food. At this time, based on AgrEvo's description of its data and analyses, the Agency considers AgrEvo's consultation on corn grain (kernels), fodder, and silage derived from corn lines containing transformation events T14 and T25 to be complete.

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